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The Short Term Effects of Manganese Toxicity on Isocitrate Dehydrogenase in Wheat & Tobacco

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THE SHORT TERM EFFECTS OF MANGANESE TOXICITY ON ISOCITRATE DEHYDROGENASE IN WHEAT AND TOBACCO

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THE SHORT TERM EFFECTS OF MANGANESE TOXICITY ON ISOCITRATE DEHYDROGENASE IN WHEAT AND TOBACCO

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Juergen W. Pfeiffer December 1988 36 pages

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The short term effects of high manganese levels (80 mg/L) on wheat and tobacco isocitrate dehydrogenase (IDH) activity were compared. The change in activity was then compared to the concentrations of manganese in the tissues.

As the time of manganese exposure increased, the fresh weight IDH activities in wheat and tobacco decreased. In contrast both control groups had similar enzyme activities every 6 hr over the 24 hr test period. The decrease in IDH activity of wheat and tobacco plants in experimental groups seemed to be related to an increase of the manganese in the leaf tissue. The slower decrease in the IDH activity of wheat and the faster decrease in the IDH activity of tobacco appeared to correspond to manganese levels in the respective tissues. It appears that wheat is able to tolerate the higher manganese concentration in the growth media better than tobacco.

INTRODUCTION

Many cash crops of farmers in the southeastern region of the United States are damaged or destroyed by manganese toxicity. Toxicity occurs because soils are more acidic and manganese has been shown to be more soluble in acidic soils (Sims and Atkinson, 1973). The application of nitrogen fertilizers increased soil acidity, leading to increased manganese uptake. The activity of the ribulose bisphosphate enzyme (RUBPCase) has been shown to decrease over longer time periods when plants were exposed to manganese toxicity (Toman et al., 1984). Anderson and Evans (1956) found during preliminary studies that manganese toxicity decreased isocitrate dehydrogenase (IDH) activity after symptoms of toxicity were seen in the plants. Miner and Sims (1983) showed that dry matter accumulation in field-grown tobacco decreased before any toxicity symptoms were seen in the plants. To appreciate the magnitude of this problem, consider the Kentucky tobacco farmers whose loss is estimated to be three to five million dollars annually.

Wheat, which is used as a winter cover crop in many of the tobacco fields, is also affected by this mechanism. Since manganese toxicity causes uncertain short term biochemical or molecular effects, this study was concerned with the following objectives:

1) Compare the short term effects of high manganese levels on wheat and tobacco,

2) Check the short term effects of high manganese levels on the activity of isocitrate dehydrogenase, and

3) Determine the concentration of manganese in leaf tissue over time.

LITERATURE REVIEW

Manganese is widely distributed in the combined state. It is the twelfth most abundant element and constitutes about 0.85% of the earth's crust. Of all the heavy metals only iron is more abundant. Manganese is one of the trace elements essential to plant and animal life. Manganese in soils is primarily dependent upon the manganese content of the parent rocks, which may have a relation to the location of those rocks or may be a considerable distance removed. The content of manganese is also effected by weathering, drainage or transportation (National Research Council, 1973). The levels of manganese found in the soil depending upon these factors can range from 500 to 900 mg per kg of soil (Bingham et al., 1979).

The availability of manganese to plants is affected by many different soil characteristics, which include the concentration of reducible manganese in the parent soil, concentrations of other divalent cations and total salts, microbial activity and the pH of the soil. A small change in any one of these soil characteristics can cause the soil to have insufficient, adequate or toxic levels of manganese for a given crop. Plants apparently absorb manganese mainly in the divalent state. Lowering the soil pH or reducing soil aeration by flooding or compacting favors the reduction of manganese to this form and thereby increases its solubility and availability to plants. The supply of

soluble manganese in equilibrium with the exchangeable manganous ion, depends on the relative importance of these two opposing reactions:

1) $Mn^{2} + 2\ OH^{-} + 1/2\ O_2 \longrightarrow MnO_2 + H_2O$

2) $MnO_2 + 4\ H^{+} + 2\ e^{-} \longrightarrow Mn^{2+} + H_2O$

The divalent manganese ion activates many enzyme reactions involved in carbohydrate breakdown and in the metabolism of organic acids, nitrogen and phosphorous. Manganese is involved in photosynthesis, where it plays an important role in maintaining chloroplast structure by being firmly bound to the lamellae of the chloroplast (Kuo and Mikkelson, 1981). The synthesis of steroids is promoted by manganese and manganese participates in stabilizing ribosome structure.

Manganese uptake may be decreased by high concentrations of calcium, magnesium, iron, ammonia ion, or other competing cations in the growth medium. In general, the manganese concentration in the plant tops is a good indicator of the manganese status of the crop (Foy et al., 1978). Even so, the manganese concentration is higher in older leaves than in younger leaves. Manganese content is also generally higher in leaves than in stems, petioles and roots. Manganese tends to accumulate in leaf margins, distal interveinal areas, leaf tips and localized areas on older leaves. Toxicity in plants is characterized by marginal chlorosis, cupping of young leaves and speckling of older leaves, which is associated with localized manganese accumulations. Necrotic spots on barley leaves can be

prevented by adding soluble silicon to the nutrient solution. The beneficial effect of silicon is attributed to a redistribution of manganese within the plant, rather than a reduction in the uptake of manganese. Other physiologic disorders associated with manganese toxicity are "crinkle leaf" of cotton, "stem streak necrosis" of Irish potato, "leaf necrosis" of tobacco and "internal bark necrosis" of apple trees. In severe cases of manganese toxicity, plant roots turn brown; this generally does not occur until after plant tops have been noticeably injured (Foy et al., 1978).

Two other factors that affect manganese uptake and toxicity are temperature and light intensity. Higher temperatures increase the uptake of manganese by plants. It has also been demonstrated that the lack of severe manganese toxicity in Maine potatoes is due to the reduced manganese uptake at low soil temperatures. Seasonal effects on manganese uptake coincided with temperature effects. Plants grown in a warm greenhouse tolerated higher manganese levels in their plant tops than those grown in the field. Some manganese tolerant plants appear to escape injury either by absorbing less manganese or by trapping the excess in the roots or other plant parts, where it is physically or chemically separated from key metabolic sites (Terry et al., 1975).

Isocitrate dehydrogenase is an important enzyme found in all organisms that undergo aerobic respiration. Most of the research has been done on bacterial and vertebrate systems, and not much is known about plant systems. This

enzyme is responsible for the conversion of isocitrate to alpha-ketoglutarate. Isocitrate is an intermediate in the Krebs' cycle, the major oxidative metabolic pathway in many organisms (Aitken and Brown, 1972). Isocitrate is also a participant in an alternate metabolic cycle, the glyoxylate shunt. In this shunt isocitrate is converted to succinate and glyoxylate by the action of the enzyme isocitrate lyase; the glyoxylate is then condensed with an acetyl-CoA to yield one molecule of malate. The malate is then decarboxylated to pyruvate, for use in glucogenesis, or for re-entry into the Krebs' cycle. The glyoxylate shunt occurs only in plants, since the isocitrate lyase enzyme is not found in animal systems. Isocitrate dehydrogenase is the metabolic supplier for the Krebs' cycle and glyoxylate shunt, but it also acts as a control point for the two pathways. It has also been suggested by Mayed and Umbarger (1962) that isocitrate dehydrogenase is connected with the control of the biosynthesis of glutamate and arginine through the Cori cycle. Kornberg and Pricer (1951) illustrated the existence of two separate forms of isocitrate dehydrogenase, differing in the required coenzyme. One form required nicotinamide adenine dinucleotide phosphate ($NADP^+$), while the other required nicotinamide adenine dinucleotide (NAD^+). The difference of cofactor requirements has been the basis for classification of isocitrate dehydrogenase. The enzyme itself can occur in two different structural forms. It can either exist as a large complex or a dimer that is made up of two smaller subgroups. Most (80-90%) of the $NADP^+$

specific activity was found in the soluble, extramitochondrial portion of the cells. It was concluded that this extramitochondrial isocitrate dehydrogenase might serve as a source of NADPH for biosynthetic processes (Lowenstein, 1961). It has since been generally accepted that the $NADP^+$ specific isocitrate dehydrogenase functions in the generation of NADPH for purposes other than the production of adenosine triphosphate (ATP), whereas the NAD^+ specific form is responsible for the control of the Krebs' cycle (Warren et al., 1966).

MATERIAL AND METHODS

Chemicals

Nicotinamide adenine dinucleotide phosphate, isocitrate substrate (DL-isocitric acid) and a manganese chloride solution ($MnCl_2$ in NaCl) were obtained from Sigma Diagnostics Supply Company. $MnCl_2 \times 4\ H_2O$ and other chemicals were on hand in the Biology Department stockroom.

Seeds

The ponca wheat seeds were donated by Southern States Farm Supply Company, Bowling Green, Kentucky. The burley tobacco seeds were supplied by Newton Farms, Hopkinsville, Kentucky.

Growth of Plants

The ponca wheat and the burley tobacco were grown in a nine to one mixture of Jiffy Mix and sand. After the wheat germinated and grew for 28 d on a 12-12 photoperiod with a day temperature of 30°C and a night temperature of 16°C, it was transplanted into four-inch plastic pots. Plants were watered with Peters Professional Soluble Plant Food, which was diluted by instructions, during germination and maturation periods. The plants were allowed to mature for two weeks, after which time they were introduced into half strength Hoagland's #2 solution (Hoagland and Arnon, 1950). The plants were allowed to adjust to their new environment for seven days. During this time the half-strength Hoagland's solution was changed every three days to prevent

bacterial or algal growth, which could have interfered with the experiments. When the 80 mg/L of manganese as manganese chloride was added to the experimental group, the photoperiod was changed to 24 hours. From then on, leaf tissue samples were obtained for various experiments every 6 hr for a 24 hr period.

Tobacco seeds were germinated and grown for 60 d under the same conditions as the wheat. The tobacco plants were also transplanted, aerated, exposed to manganese and sampled in the same way as the wheat plants.

Isocitrate Dehydrogenase Extraction and Assay

Fresh plant tissue (0.70 g) was macerated with a mortar and pestle in 5 mL of 0.5 M Tris-(hydroxymethyl)-aminomethane buffer in the cold. The resulting mixture was centrifuged at 20,000 x g for 10 min at 2°C in a Beckman centrifuge (Anderson and Evans, 1956). The force of 20,000 x g was selected because the procedure allowed both the cytoplasmic and mitochondrial isocitrate dehydrogenase enzymes to be extracted. The crude enzyme extract was removed from the centrifuge tube and placed on ice to prevent loss of enzyme activity. The extract was assayed by incubating 0.5 mL of crude enzyme extract with 1.2 mL of isocitrate substrate (10 uM/mL) and two drops of manganese chloride (0.10 M/L). The reaction was initiated by the addition of 0.5 mL of $NADP^+$ solution (1.0 mg/2 mL). The increase in absorbance at 340 nm was determined every minute for 6 min with a Gilford Response and a Milton Roy Spectronic 21 spectrophotometer. The enzyme activity was

directly related to the increased absorbance at 340 nm over the 6 min time period, because isocitrate dehydrogenase reduced $NADP^+$ to NADPH.

Manganese Determination by Atomic Absorption Spectroscopy

Fresh plant tissue (1.0 g) was dried in a hot air oven at 30°C for four days. After that time period in the drying oven the plant material was reweighed and digested in an acid solution composed of nine parts nitric acid and one part perchloric acid. The digestion occurred when the mixture was heated over a boiling water bath for 12 hours. The remaining liquid was evaporated slowly to dryness and the residue was redissolved in 5 mL of 1 N hydrochloric acid. This mixture was then heated for 20 min at 100°C, with occasional agitation. The resulting solution was assayed for manganese using a Perkin Elmer atomic absorption spectrophotometer.

Protein Determination of Enzyme Extracts

The Bio-Rad dye concentrate was diluted with four equal volumes of deionized water. The mixture was filtered through a double layer of cheesecloth to remove extraneous particles which could have otherwise interfered with the spectrophotometric assay. After filtering the dye solution, 5 mL of the diluted dye were added to 0.1 mL of crude IDH extract and mixed. The mixture was set aside for 15 min to allow the complex formation to occur. The absorbance of this complex was determined at 595 nm on a Gilford Response spectrophotometer and compared to the standard protein

concentration curve, which was determined using BSA as a standard protein.

RESULTS

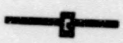
Fresh Weight IDH Activity of Tobacco

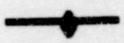
When IDH activities of fresh weight control and experimental groups were compared by spectrophotometric technique, a decrease or increase in the slope of the line corresponded to a decrease or increase in enzyme activity. The comparison of control tobacco plants every 6 hr over a 24 hr period showed approximately the same enzyme activity on the basis of fresh weight. The enzyme activities were determined by a comparison of the slopes every 6 hr over the 24 hr time period; the high was 122 units and the low was 78 units (Fig. 1). Slopes were determined by the change in absorbance over the 6 min time period that the isocitrate dehydrogenase was assayed and were expressed as units of enzyme activity. After the tobacco plants were exposed to 24 hr of manganese (80 mg/L), there was a noticeable difference in enzyme activity when compared to the control group. Following a slight increase in activity after 6 hr of exposure, the activity of the enzyme decreased dramatically over the 24 hr period. After 24 hr, the slope of the line approached zero. The high slope of 101 units occurred after 6 hr and the low slope of 27 units occurred after 24 hr (Fig. 2).

The units of IDH activity per g fresh weight of tobacco leaf tissue were calculated from Figures 1 and 2 and are presented in Table 1. The units of enzyme activity per g

FIGURE 1: Absorbance of control tobacco enzyme assay mixture/70 mg fresh weight over a 6 min time period, where each point represents the average of 8 replications.

where


represents plants grown in half strength Hoagland solution for 6 hr.


represents plants grown in half strength Hoagland solution for 12 hr.


represents plants grown in half strength Hoagland solution for 18 hr.


represents plants grown in half strength Hoagland solution for 24 hr.

Tobacco/Control

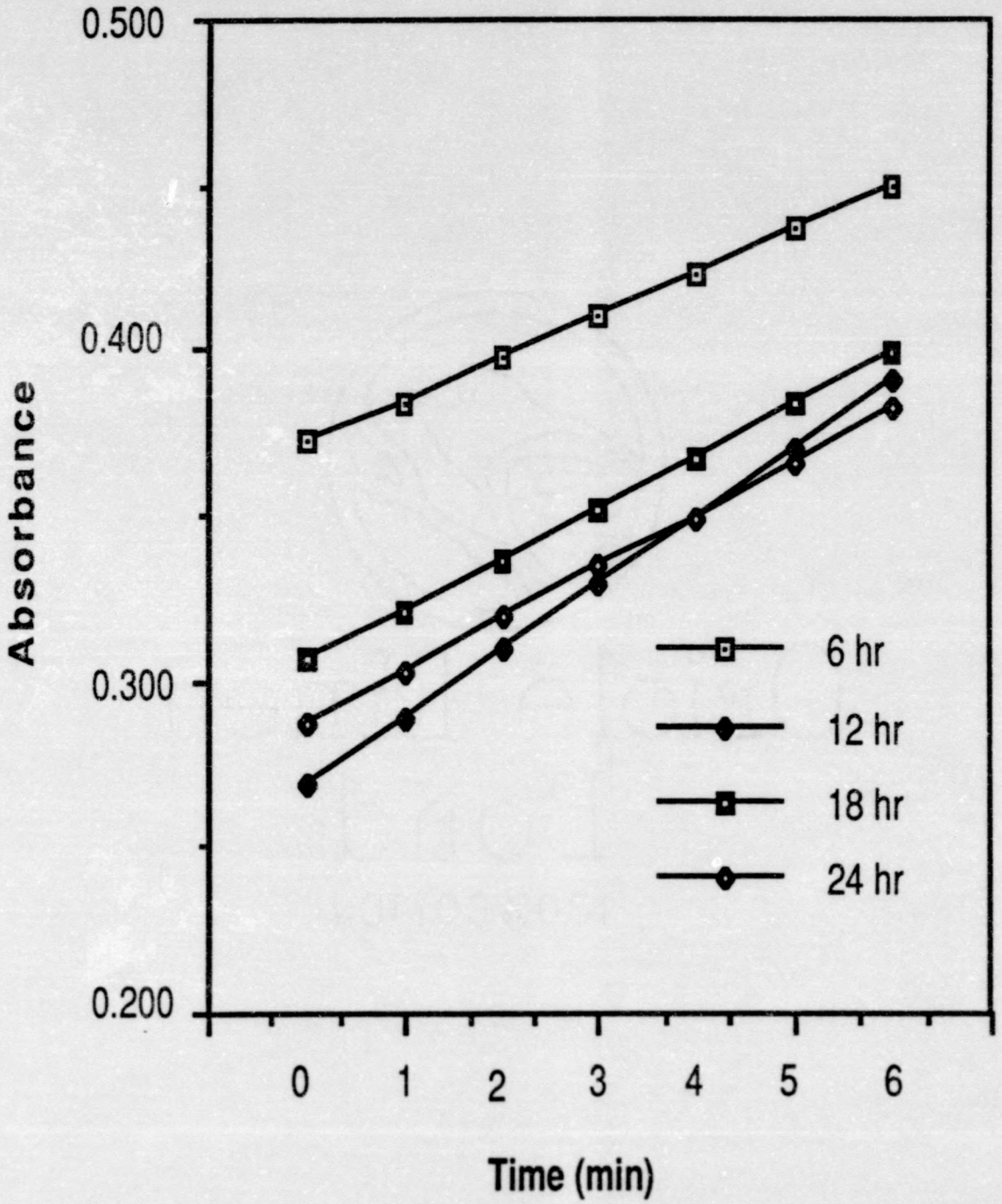
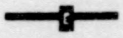


FIGURE 2. Absorbance of experimental tobacco enzyme assay mixture/70 mg fresh weight over a 6 min time period, where each point represents the average of 8 replications.

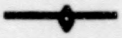
where



represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 6 hr.

represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 12 hr.

represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 18 hr.



represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 24 hr.

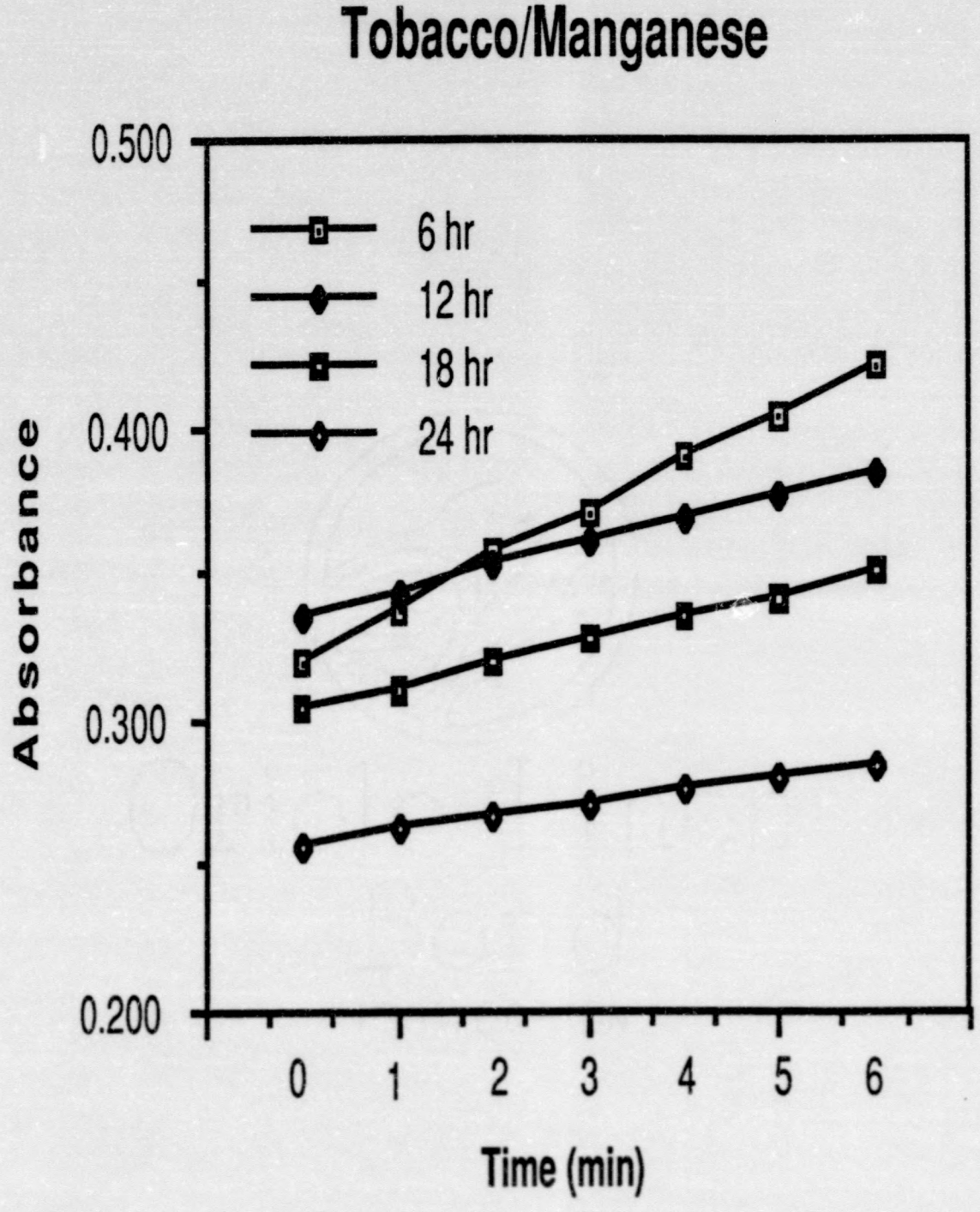

Tobacco/Manganese
0.500
0.400
0.300
0.200
Absorbance
6 hr
12 hr
18 hr
24 hr
0
1
2
3
4
5
6
Time (min)

TABLE 1

Units of enzyme activity/g fresh wt of tobacco leaf tissue.

	Experimental	Control
6 hr	1442	1442
12 hr	685	1742
18 hr	685	1328
24 hr	385	1214

fresh wt of tobacco leaf tissue, showed an overall decrease as manganese exposure to the tissue increased. After 6 hr, the experimental group showed a small increase in activity. When the time increased to 12 hr, the activity of the experimental group was reduced by 50 percent, while the control group remained about the same. At the 18 hr mark of manganese exposure, there seemed to be no more inhibitory effects of the metal on the isocitrate dehydrogenase. However, the activity of the experimental group decreased to 385 units after 24 hr, one-half of what it was 6 hr before. The 385 units of activity were approximately one-fourth the activity of the control group (1214 units) (Table 1).

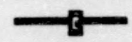
Fresh weight IDH Activity of Wheat

The comparison of IDH activity in wheat control plants over the 24 hr period showed that there was not much difference in activity on the basis of fresh weight. The activity was determined by a comparison of the slopes every 6 hr over the 24 hr time period, where the high was 141 units and the low was 112 units (Fig. 3). The wheat experimental group was exposed to manganese (80 mg/L) for 24 hours. Following a prominent increase in enzyme activity after 6 hr of treatment, the activity continuously decreased over the remainder of the 24 hr period of exposure to high levels of manganese. The high slope of 203 units occurred after 6 hr and the low slope of 81 units occurred after 24 hr (Fig. 4).

The units of IDH activity per g fresh wt of wheat leaf tissue were calculated from Figures 3 and 4 and are

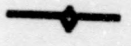
FIGURE 3: Absorbance of control wheat enzyme assay mixture/70 mg fresh weight over a 6 min time period, where each point represents the average of 6 replications.

where


represents plants grown in half strength Hoagland solution for 6 hr.


represents plants grown in half strength Hoagland solution for 12 hr.


represents plants grown in half strength Hoagland solution for 18 hr.


represents plants grown in half strength Hoagland solution for 24 hr.

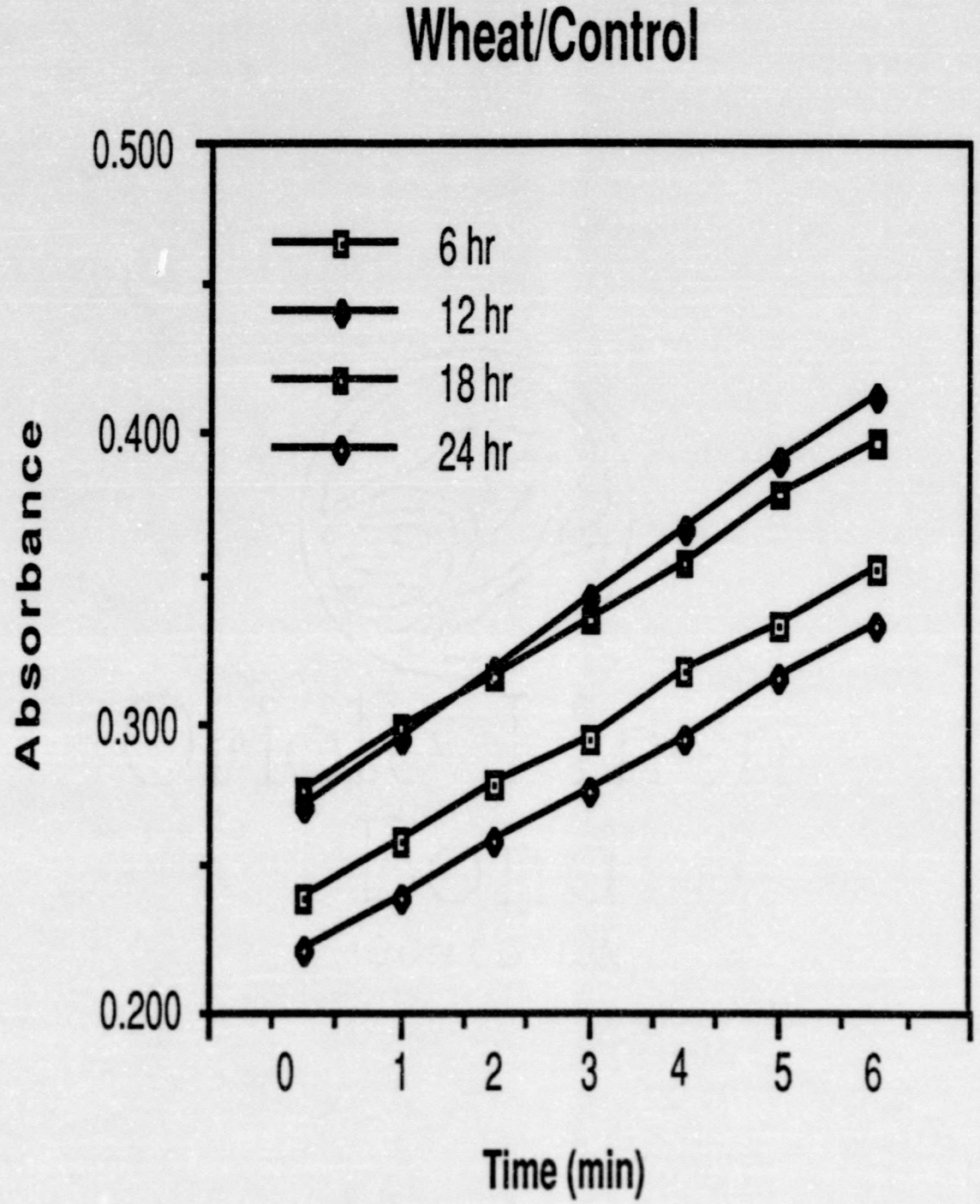
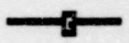

Wheat/Control
0.500
0.400
0.300
0.200
Absorbance
6 hr
12 hr
18 hr
24 hr
0
1
2
3
4
5
6
Time (min)

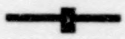
FIGURE 4. Absorbance of experimental wheat enzyme assay mixture/70 mg fresh weight over a 6 min time period, where each point represents the average of 6 replications.

where



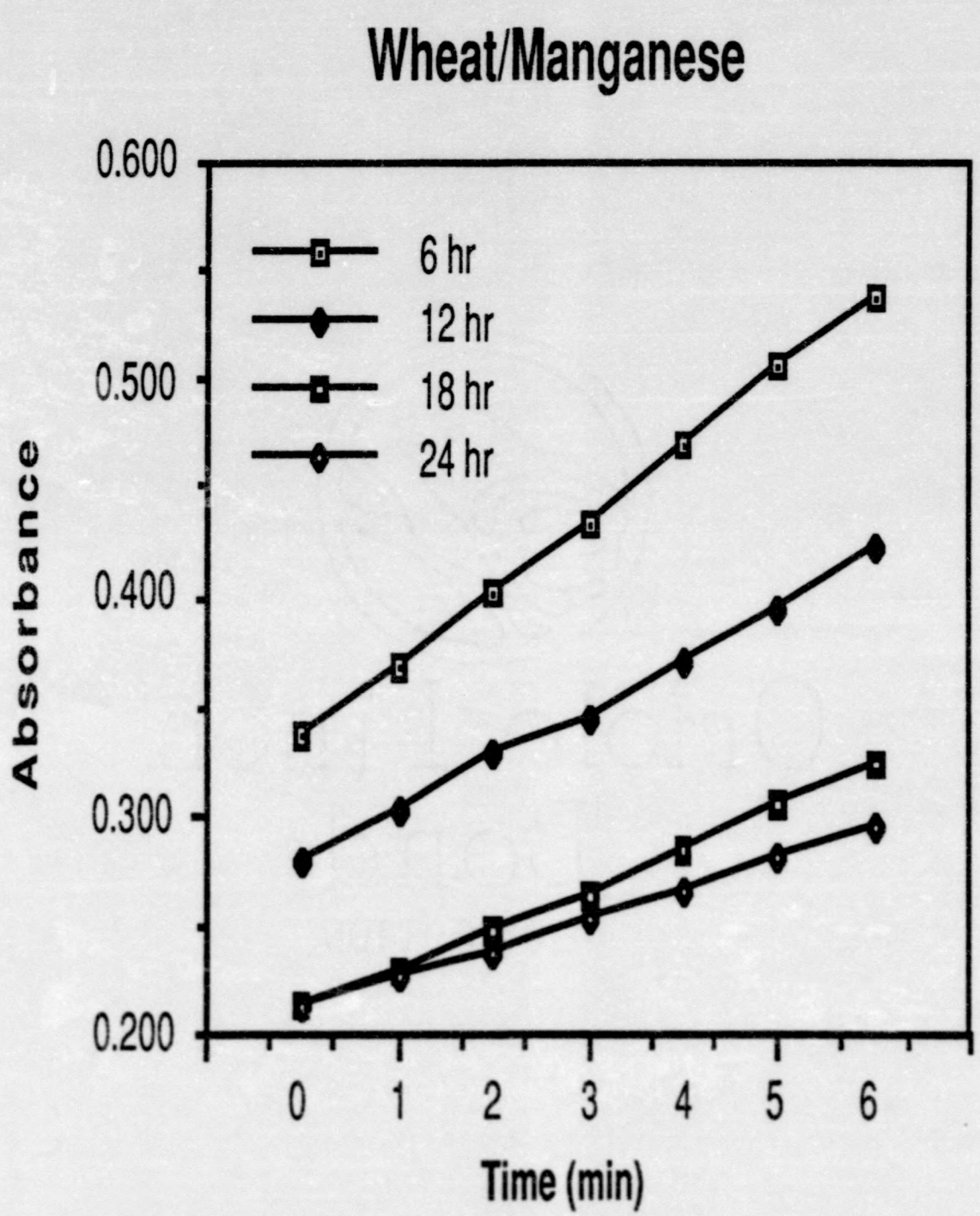
represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 6 hr.

represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 12 hr.



represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 18 hr.

represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 24 hr.


Wheat/Manganese
0.600
0.500
0.400
0.300
0.200
Absorbance
6 hr
12 hr
18 hr
24 hr
0
1
2
3
4
5
6
Time (min)

presented in Table 2. As the time of manganese exposure increased, overall the units of enzyme activity per g fresh wt of wheat tissue decreased. This decrease in the experimental group followed a sharp increase in the activity after 6 hr, where the experimental group activity was 2900 units and the control group activity was only 1614 units. During the remaining 18 hr of the test period, the experimental group activity decreased in a steady manner, while the control remained approximately the same over this time period. Even with the sharp increase in IDH activity during the first 6 hr of manganese exposure, the experimental group still had over 400 units less activity than the control group after 24 hr (Table 2).

Protein IDH Activity of Tobacco

When enzyme activity experiments are conducted, it is customary to determine protein concentration of the extract allowing the activity to be expressed per mg protein. The enzyme activity determined in the fresh weight experiments was divided by the protein content of the crude extract (0.5 mL) resulting in enzyme activity/mg protein. An increase or decrease in the slope of the line corresponds to an increase or decrease in IDH activity. The comparison of tobacco control plants every 6 hr over a 24 hr period showed approximately the same activity on a protein basis. The IDH activities were determined by the comparison of the slopes every 6 hr over the 24 hr time period, where the high was 90 units and the low was 50 units (Fig. 5). After the experimental tobacco group was exposed to 24 hr of high

TABLE 2

Units of enzyme activity/g fresh wt of wheat leaf tissue.

	Experimental	Control
6 hr	2900	1614
12 hr	2042	2014
18 hr	1585	1714
24 hr	1142	1600

FIGURE 5: Absorbance of control tobacco enzyme assay mixture/mg protein over a 6 min time period, where each point represents the average of 4 replications.

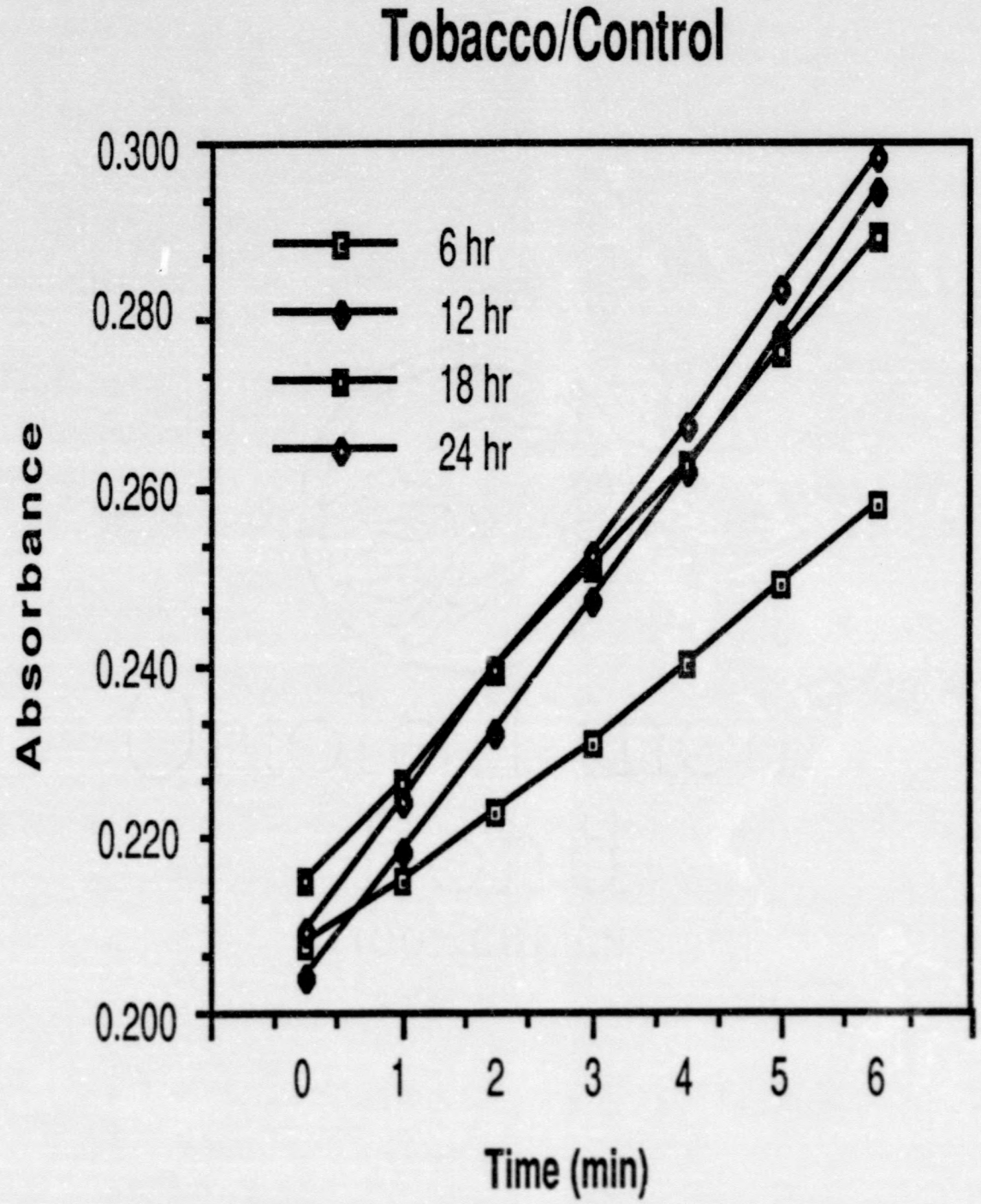
where


represents plants grown in half strength Hoagland solution for 6 hr.


represents plants grown in half strength Hoagland solution for 12 hr.


represents plants grown in half strength Hoagland solution for 18 hr.


represents plants grown in half strength Hoagland solution for 24 hr.


Tobacco/Control
0.300
0.280
0.260
0.240
0.220
0.200
Absorbance
6 hr
12 hr
18 hr
24 hr
0
1
2
3
4
5
6
Time (min)

manganese levels (80 mg/L), the IDH activity showed a large decrease. The decrease of IDH activity was steady over the entire 24 hr period, starting with a high of 73 units after 6 hr and ending with a low of 11 units after 24 hours. The slope of the line approached zero after 24 hr (Fig. 6). The data of the enzyme activity/mg protein (slopes) in Table 3 illustrate the overall trends between experimental and control tobacco groups.

Protein IDH Activity of Wheat

The comparison of control wheat plants every 6 hr over a 24 hr time period showed approximately the same activity on a protein basis. The enzyme activities were determined by the comparison of the slopes every 6 hr over the 24 hr time period, where the high was 96 units and the low was 80 units (Fig. 7). After the experimental wheat group was exposed to only 6 hr of high manganese levels (80 mg/L), the IDH activity showed a large increase (199 units). Over the remaining 18 hr the IDH activity decreased continuously to 97 units after 24 hr of exposure (Fig. 8). The data of the enzyme activity/mg protein (slopes) in Table 4 illustrate the overall trends between control and experimental wheat groups.

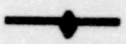
Manganese Concentration Determination

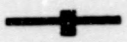
When manganese concentrations of control tobacco plants were compared, they ranged from 50-70 micro-grams per g dry wt of leaf tissue. After only 6 hr of exposure to high manganese concentrations (80 mg/L), the amount of the metal in the tissue increased several fold.

FIGURE 6: Absorbance of experimental tobacco enzyme assay mixture/mg protein over a 6 min time period, where each point represents the average of 4 replications.

where


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 6 hr.


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 12 hr.


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 18 hr.


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 24 hr.

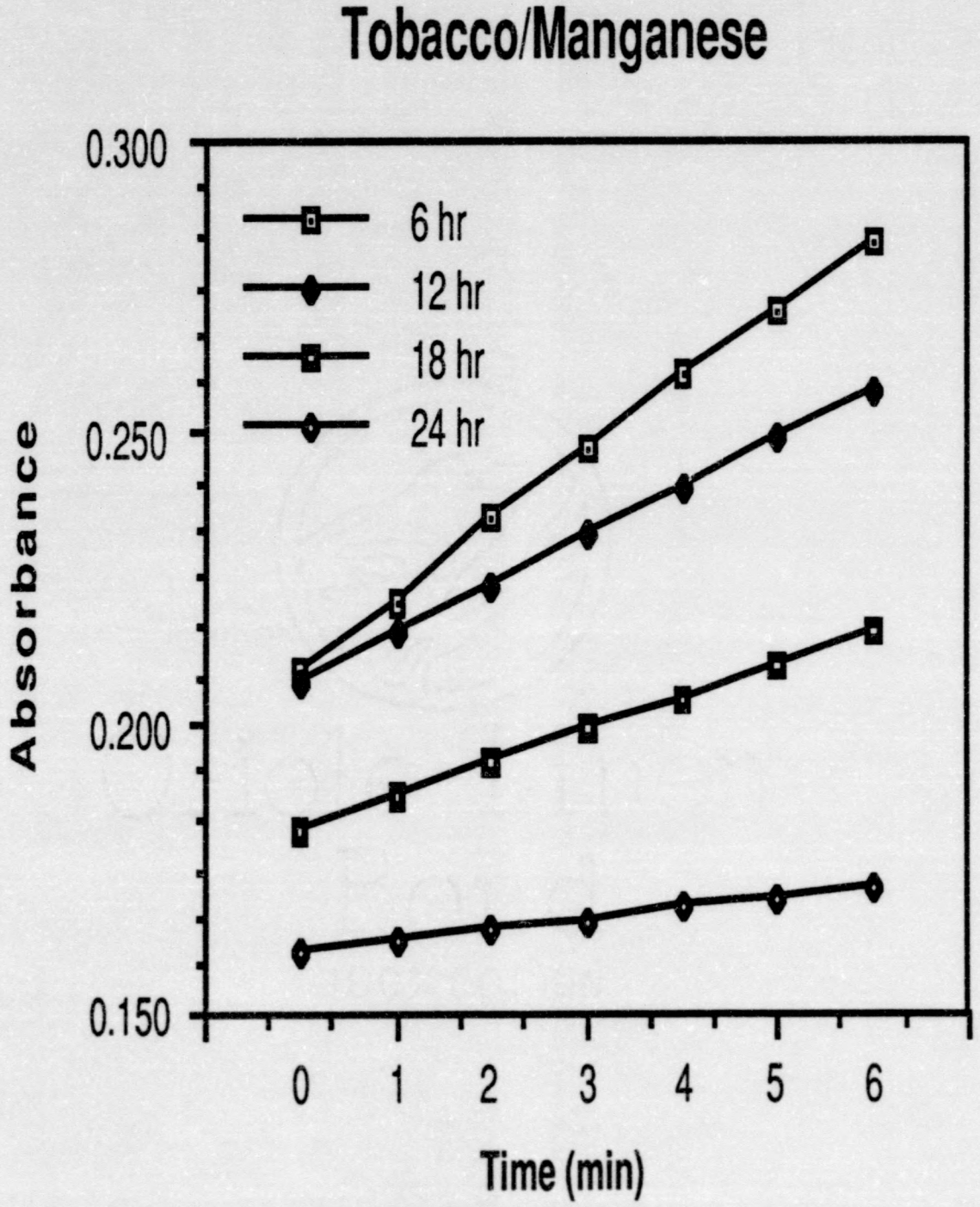

Tobacco/Manganese
0.300
0.250
0.200
0.150
Absorbance
6 hr
12 hr
18 hr
24 hr
0
1
2
3
4
5
6
Time (min)

TABLE 3

Units of enzyme activity/mg protein (slope) of isocitrate dehydrogenase extract from tobacco leaf tissue every 6 hr over a 24 hr period.

	Experimental	Control
6 hr	73	50
12 hr	50	90
18 hr	35	74
24 hr	11	89

FIGURE 7: Absorbance of control wheat enzyme assay mixture/mg protein over a 6 min time period, where each point represents the average of 3 replications.

where


represents plants grown in half strength Hoagland solution for 6 hr.


represents plants grown in half strength Hoagland solution for 12 hr.


represents plants grown in half strength Hoagland solution for 18 hr.


represents plants grown in half strength Hoagland solution for 24 hr.

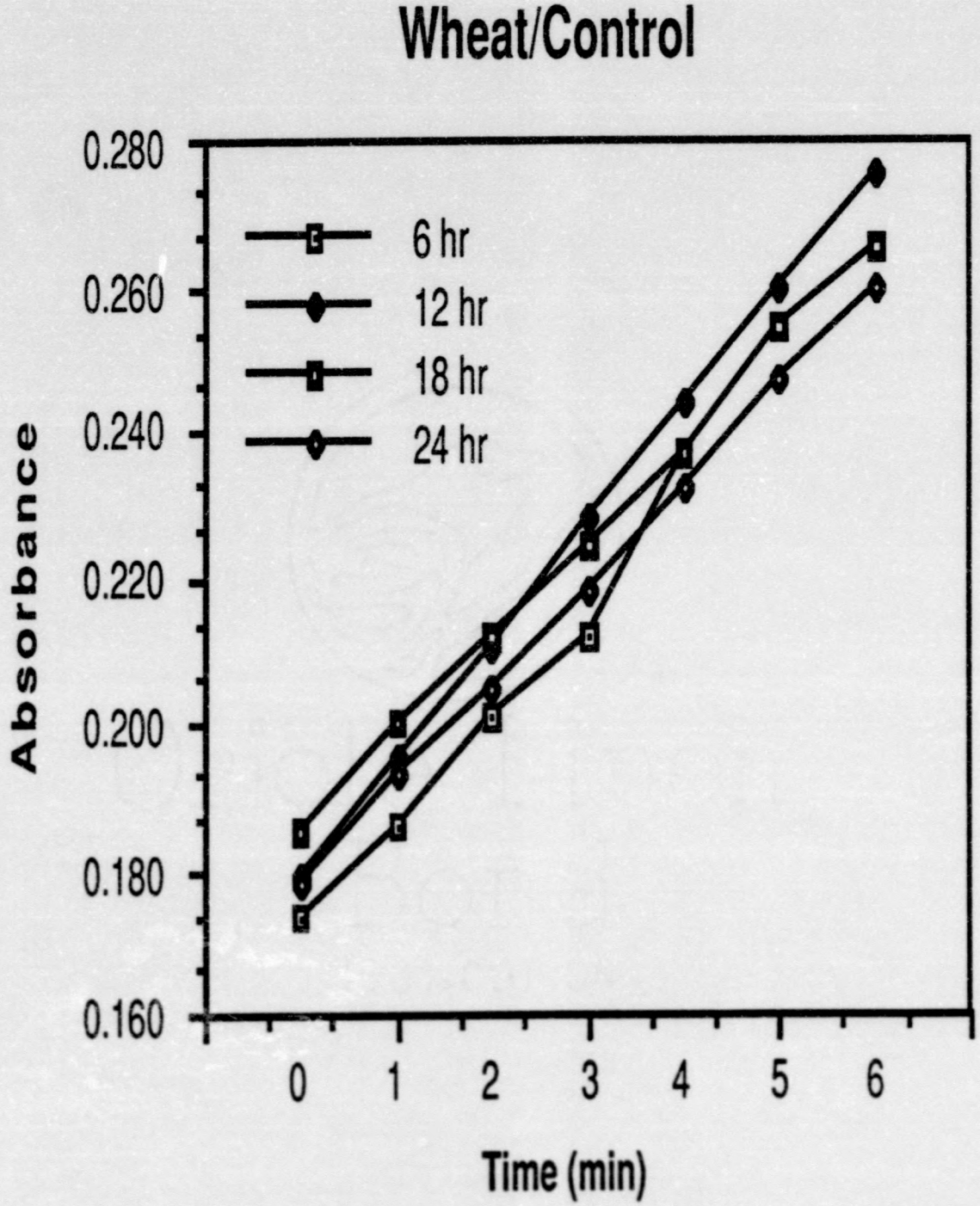
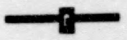

Wheat/Control
0.280
0.260
0.240
0.220
0.200
0.180
0.160
Absorbance
6 hr
12 hr
18 hr
24 hr
0
1
2
3
4
5
6
Time (min)

FIGURE 8: Absorbance of experimental wheat enzyme assay mixture/mg protein over a 6 min time period, where each point represents the average of 3 replications.

where


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 6 hr.


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 12 hr.


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 18 hr.


represents plants grown in half strength Hoagland solution and 80 mg/L manganese for 24 hr.

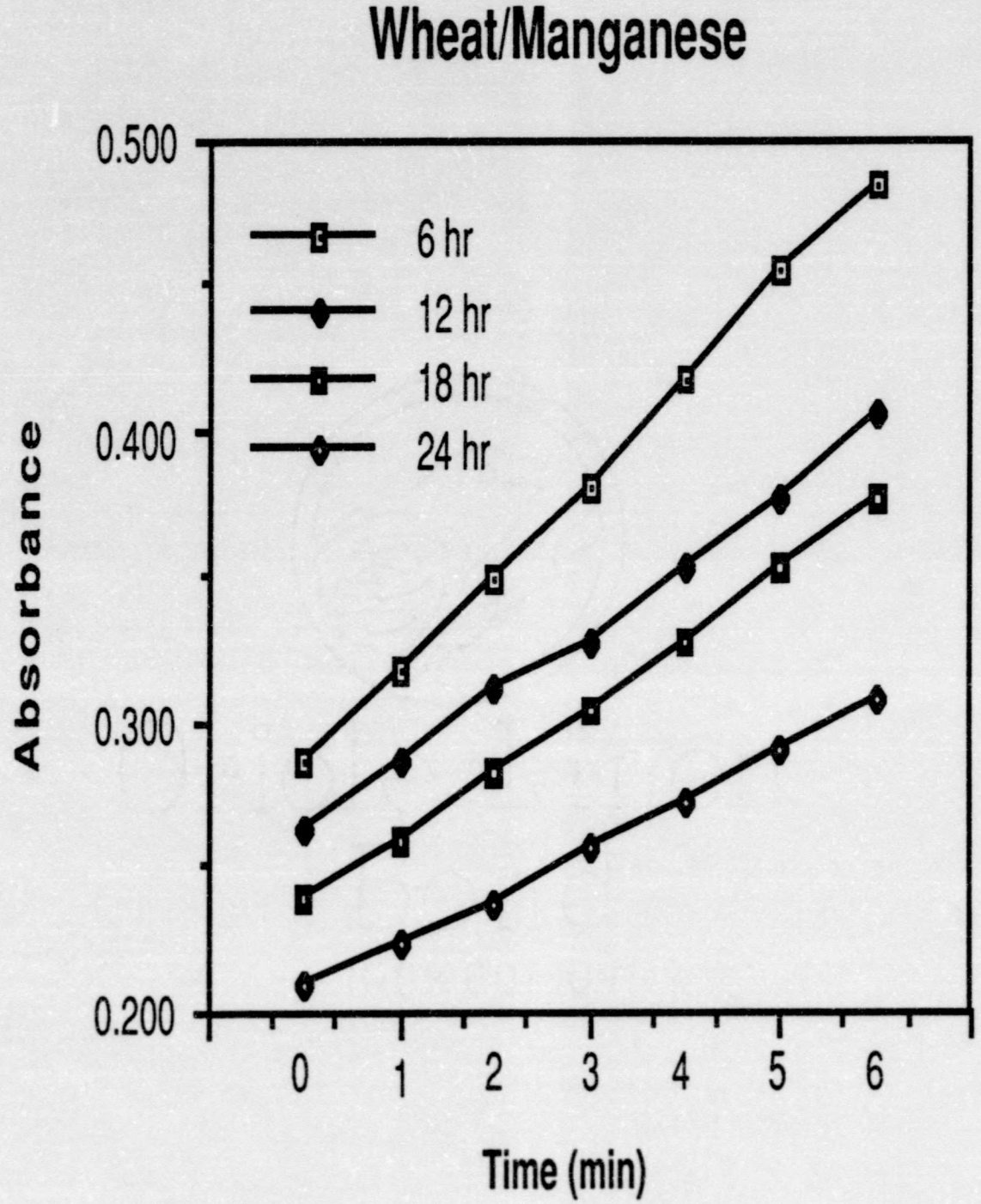

Wheat/Manganese
0.500
0.400
0.300
0.200
Absorbance
6 hr
12 hr
18 hr
24 hr
0
1
2
3
4
5
6
Time (min)

TABLE 4

Units of enzyme activity/mg protein (slope) of isocitrate dehydrogenase extract from wheat leaf tissue every 6 hr over 24 hr period.

	Experimental	Control
6 hr	199	92
12 hr	142	96
18 hr	137	80
24 hr	97	82

The increase continued through 12 hr, however, during the next 6 hr there was a slight decrease of manganese in the tobacco leaf tissue. This decrease was followed by a rather large increase during the remaining 6 hr (Table 5).

When manganese concentrations of control wheat plants were compared, they were found to be between 50-70 micrograms per g dry weight of leaf tissue. In the experimental group the manganese increased continuously over the first 18 hours. When the experimental group was examined over the last 6 hr of the 24 hr test period, there was a slight decrease in the concentration of the metal in the tissue (Table 6).

TABLE 5

Micrograms of manganese/g dry wt of experimental tobacco leaf tissue.

Exp.	6 hr	12 hr	18 hr	24 hr
1	247.4	536.9	493.1	1367.7
2	679.1	957.5	808.3	1124.1
3	363.2	647.6	563.0	1183.3
4	518.8	808.2	699.8	1254.0
5	312.0	638.1	506.6	1333.9
6	630.9	893.7	701.9	1109.2
7	700.3	986.8	871.4	1398.6
8	301.6	662.2	600.2	1089.8

Manganese concentration of control tobacco tissue was found to be 50-70 micrograms/gram.

TABLE 6

Micrograms of manganese/g dry wt of experimental wheat tissue.

Exp.	6 hr	12 hr	18 hr	24 hr
1	88.9	222.0	353.5	317.2
2	172.2	177.8	257.8	206.5
3	153.1	200.8	298.3	256.4
4	107.7	163.0	231.1	196.6
5	96.5	193.0	277.9	244.2
6	125.5	181.0	269.3	223.2

Manganese concentration of control wheat tissue was found to be 50-70 micrograms/gram.

DISCUSSION

Isocitrate dehydrogenase (IDH) occurs in three different forms, the NAD^+-specific, the $NADP^+$-specific, and a dimeric form, in which the enzyme is actually a complex made up of the two subgroups (Chung and Franzen, 1969). It has also been reported that all isocitrate dehydrogenases, including the $NADP^+$-specific form, have divalent cation dependence. Manganese was the only metal used in this study. The full spectrum of metal usage by this enzyme was explored by Dedhia (1973). He demonstrated the ability of $NADP^+$-specific IDH to utilize the numerous metals, including mercury, which is usually considered to be inhibitory to enzyme catalyzed reactions. The present study indicated that manganese was not only necessary for isocitrate dehydrogenase activity in wheat and tobacco, but it also decreased the enzyme activity over a longer time period. This decrease in activity was also shown by Omran and Dennis (1971), when the activity of the enzyme decreased over time when exposed to heavy metals.

When fresh weight IDH activities and protein IDH activities of wheat and tobacco were compared they both showed approximately the same overall trends. The control groups of both the wheat and tobacco had similar slopes over the 24 hr test period. In both wheat and tobacco, the control groups showed a slight increase in IDH activities after 12 hours. This increase could have been stimulated by

the change to a 24 hr photoperiod, a point for future investigation.

The experimental groups of both wheat and tobacco showed an increase in enzyme activity after 6 hr of exposure to manganese. After that increase, the activity continued to decrease for the remaining 18 hours. This decrease in enzyme activity could have been caused either by the metal blocking the active site of the enzyme or by separating the enzyme complex, thus rendering the enzyme inactive.

The decrease in IDH activity of experimental wheat and tobacco groups seemed to be related to an increase of the manganese in the plant tissue. The slower decrease in IDH activity of wheat and the faster decrease in IDH activity of tobacco appeared to correspond to manganese levels in the respective tissues. The concentration of manganese in experimental tobacco tissue was much higher than the concentration of manganese in experimental wheat tissue. It appears that wheat is able to tolerate the higher manganese concentration in the growth media better than tobacco. Wheat may be able to tolerate manganese better in the environment, because the uptake of the metal is much slower and it may have more effective carriers to move it out of the leaf tissue and relocate the manganese to other plant tissues. This investigation showed both wheat and tobacco decreased in manganese concentrations over some 6 hr time period in the 24 hr test period. These decreases indicated that carriers were involved in translocating manganese to other plant tissues. Since tobacco may not be as efficient

in carrier use and selective uptake it had much higher concentrations of the metal in the leaf tissue. These high levels of manganese occurred even though the concentration in the control plants of the wheat and tobacco were the same. Therefore, it appears that farmers using wheat as a cover crop on a high manganese soil could expect a good crop and may be removing some manganese from the soil, thus allowing a better tobacco crop.

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VITA

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